

Recovery of *Mycobacterium bovis* from Soft Fresh Cheese Originating in Mexico[▽]

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Recent outbreaks of human tuberculosis in the United States caused by *Mycobacterium bovis* have implicated cheese originating in Mexico as a source of these infections. A total of 203 samples of cheese originating in Mexico were cultured, and *M. bovis* was recovered from one specimen. Therefore, *M. bovis* can be recovered from cheese and may be a source of human infections.

Bovine tuberculosis, caused by *Mycobacterium bovis*, is a zoonotic disease that also affects humans. Although people are generally infected through the inhalation of droplet nuclei, a significant proportion of human cases involve extrapulmonary tuberculosis, presumably caused by the consumption of nonpasteurized milk or dairy products (23). Indeed, milk pasteurization requirements in the United States were developed to prevent many food-borne infections, including tuberculosis, botulism, and scarlet fever, caused by consuming contaminated milk or dairy products (5). With the implementation of strict pasteurization requirements and a mandatory control program for bovine tuberculosis in live animals, the incidence of *M. bovis* infections in cattle in the United States has decreased to an all-time low of less than 0.001% (1). Consequently, human cases of *M. bovis* infections in the United States have also declined (23). However, several reports have shown an elevated incidence of human tuberculosis due to *M. bovis* in certain regions of the United States (2, 7, 14). For example, a study in San Diego County, CA, found that 129 of 1,931 (6.7%) culture-positive tuberculosis cases in the County were due to *M. bovis* (14). A similar epidemiologic investigation in New York City also reported that 1% of culture-positive tuberculosis cases in this area were due to *M. bovis* (4). In both reports, patients of Hispanic ethnicity were especially at risk and approximately one-third of the cases occurred in children. In both instances, epidemiologic investigations indicated that the consumption of unpasteurized dairy products, including soft fresh cheese originating

in Mexico may have accounted for these cases (4). Therefore, to investigate this possibility, a survey for the presence of *M. bovis* in fresh cheese products entering the United States from Mexico was initiated as a collaborative project between USDA-National Veterinary Services Laboratories (NVSL) and the California Animal Health and Food Safety Laboratories.

A total of 203 cheese samples were collected from travelers entering California at the United States Customs and Border Protection Port in San Ysidro, San Diego, CA, from March through August 2005. All cheese samples had been purchased by individuals for private consumption and were being imported through noncommercial channels. Thus, whether these products were derived from pasteurized or unpasteurized milk is unknown. These samples were shipped to USDA-NVSL for mycobacterial culture. For this, 5-g portions of cheese were weighed, aseptically transferred into a sterile stomacher bag containing 45 ml of sterile 2% sodium citrate, and homogenized in a stomacher (model no. 80 lab blender; Seward Laboratory, London, England) for 2 min. The bag was then heat sealed and submerged in a 37°C water bath for 1 h to liquefy the specimen. This suspension was then aseptically transferred into a sterile 50-ml centrifuge tube for ease of handling. The cheese suspension was decontaminated using the *N*-acetyl-L-cysteine-NaOH method as previously described (20). For this, 10 ml of the homogenized sample was mixed with 10 ml of digestant consisting of sterile 0.05 M trisodium-citrate, 2% (wt/vol) sodium hydroxide, and 0.5% (wt/vol) *N*-acetyl-L-cysteine. The mixture was vigorously shaken for 20 s and allowed to stand at room temperature for 15 min. This mixture was then neutralized with 30 ml of 0.067 M phosphate buffer and centrifuged at 5,000 × *g* for 15 min at 10°C. After removal of the supernatant, 0.5-ml aliquots of the remaining pellet were inoculated into both BACTEC 12B and BBL MGIT 960 liquid

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TABLE 1. Recovery of *Mycobacterium* species from fresh cheese originating in Mexico

Sample ID	Origin ^a	Epidemiology ^b	<i>Mycobacterium</i> species recovered	Type of cheese ^c
05-9001	Cheese	Baja California	<i>M. fortuitum</i> complex	Hard grating
05-9019	Cheese	Baja California	<i>M. fortuitum</i>	Soft fresh
05-9040	Cheese	Baja California	<i>Mycobacterium</i> sp. ^d	Soft fresh
05-9363	Cheese	Baja California	<i>M. fortuitum</i>	Soft fresh
05-9389	Cheese	Baja California	<i>M. fortuitum</i>	Soft fresh
05-9392	Cheese	Baja California	<i>M. moriokaense</i>	Soft fresh
05-9393	Cheese	Baja California	<i>M. bovis</i>	Soft fresh
05-10179	Cheese	Baja California	<i>M. fortuitum</i>	Soft fresh
05-10181	Cheese	Baja California	<i>M. fortuitum</i>	Soft fresh
05-10248	Cheese	Baja California	<i>M. fortuitum</i>	Semihard

^a Original diagnostic source of mycobacterial isolate.

^b State in Mexico that the diagnostic specimen originated from, based on concurrent epidemiological information.

^c Classification of cheese, based on visual appearance, recovered from travelers entering the United States from Mexico.

^d Identified as a *Mycobacterium* species most closely resembling *M. moriokaense*.

media (Becton Dickinson Diagnostic Systems, Sparks, MD). Each BACTEC 12B bottle was supplemented with 0.2 ml of BACTEC PANTA PLUS. A total of 6.3 mg/ml of erythromycin was also added to help eliminate overgrowth by contaminants. Similarly, each BBL MGIT mycobacteria growth indicator tube was supplemented with 0.8 ml of BBL MGIT growth supplement-BBL MGIT PANTA antibiotic mixture (Becton Dickinson) and 7.0 mg/ml of erythromycin. Specimens were incubated at 37°C and monitored for growth for a total of 6 weeks according to the manufacturer's protocols. Positive identification of *M. bovis* was performed using an AccuProbe *Mycobacterium tuberculosis* complex culture identification test kit (Gen-Probe, San Diego, CA) and negative biochemical reactions for niacin and nitrate (8). Genetic confirmation of *M. tuberculosis* complex isolates as *M. bovis* was accomplished using a PCR-based typing method targeting the *M. tuberculosis* complex chromosomal region-of-difference deletion loci, as described previously (9).

Of the 203 cheese samples cultured, 10 (4.9%) were positive for bacteria belonging to the genus *Mycobacterium*, with one isolate being identified as *M. bovis*. The ability to recover *M. bovis* from raw milk is well documented, especially from milk obtained from cattle residing in areas with a high regional prevalence of bovine tuberculosis (11, 13). In Mexico, the incidence of bovine tuberculosis varies by region, with beef cattle in the northernmost states having the lowest prevalence at less than 2% (21). However, the prevalence of *M. bovis* in dairy cattle in Mexico is significantly higher, with an estimated infection rate in this population of 16 to 17% (15, 17). Using partial 16S rRNA gene sequencing (12) and standard biochemical tests (8), seven mycobacterial strains were identified as *M. fortuitum* or *M. fortuitum* complex, one was identified as *M. moriokaense*, and one was identified as a *Mycobacterium* species resembling *M. moriokaense* (Table 1). The presence or absence of mycobacteria could not be confirmed for 6 of the cheese cultures due to overgrowth by contaminants, and the remaining 187 cultures were negative for acid-fast bacteria. The 10 samples from which *Mycobacterium* species was recovered comprised several cheese varieties, including a hard-grating type, a semi-hard type, and several types of soft cheese (Table 1). The

recovery of nontuberculous mycobacteria from cheese samples in this study is consistent with other reports that describe the recovery of various *Mycobacterium* species, including *M. fortuitum*, from raw milk obtained from dairy cattle (11, 13). Although not as severe a public health concern as *M. bovis* bacteria, *M. fortuitum* complex bacteria are opportunistic pathogens and are implicated in various clinical diseases, especially in humans with immunocompromised immune systems (3). Because no history is available regarding the production of the cheese obtained during this survey, environmental sources of these mycobacteria cannot be ruled out due to the contamination of milk during either handling or processing.

Drug susceptibility testing was performed on the sole *M. bovis* isolate by using BACTEC 12B medium and the radiometric modified proportion method (BACTEC 460; Becton-Dickinson) (8) with the following drugs (and concentrations); streptomycin (2 µg/ml), isoniazid (0.1 µg/ml), rifampin (2 µg/ml), ethambutol (2.5 µg/ml), and pyrazinamide (100 µg/ml). This isolate was susceptible to all antibiotics tested except pyrazinamide, to which *M. bovis* is intrinsically resistant (22). Because the transmission of drug-resistant bacterial pathogens from animals to humans is a significant public health concern, an additional 11 random *M. bovis* isolates from the NVSL culture collection, obtained from cattle with epidemiological links to Mexico, were also tested for antimicrobial susceptibility (data not shown). All strains of *M. bovis* were pansusceptible to the antibiotics tested, with the exception of pyrazinamide. Although a comprehensive survey of antibiotic resistance in *M. bovis* field isolates was beyond the scope of this survey, it appears that resistance to antituberculosis drugs occurs infrequently in cattle from Mexico. This lack of antibiotic resistance is consistent with federal bovine tuberculosis control programs in both the United States and Mexico, which require that all infected animals be depopulated rather than treated for infection. However, an antibiotic-susceptible phenotype may be associated with diverse genotypes and thus be unrelated, requiring caution in the interpretation of these antibiograms.

To determine whether the *M. bovis* strain recovered from the cheese sample was related to other *M. bovis* strains seen in cattle from North America, this isolate was genotyped using the standard NVSL protocol of spoligotyping, IS6110 restriction fragment length polymorphism (RFLP), and polymorphic GC-rich repetitive sequence (PGRS)-RFLP, as described elsewhere (10, 18), with the following modifications for the IS6110 RFLP. For this technique, *M. bovis* genomic DNA was digested with 10 U of PvuII and a 445-bp IS6110 probe, spanning the PvuII restriction site and thus producing two bands for each copy of IS6110 present, was utilized. To generate this probe, a portion of the IS6110 element was PCR amplified using the primers 445R (5'-CGG ACA GGC CGA GTT GGT CAT C-3') and 445L (5'-GAC CAC GAC CGA AGA ATC CGC TG-3').

As seen in Fig. 1, the *M. bovis* isolate recovered from the cheese originating in Mexico is highly similar to three other *M. bovis* isolates recovered from cattle entering the United States from Mexico. Although this spoligotype pattern is identical for all of the isolates reported here, it does not match any other spoligotypes reported previously for cattle from Mexico (6, 16). However, it should be noted that those previous studies fo-

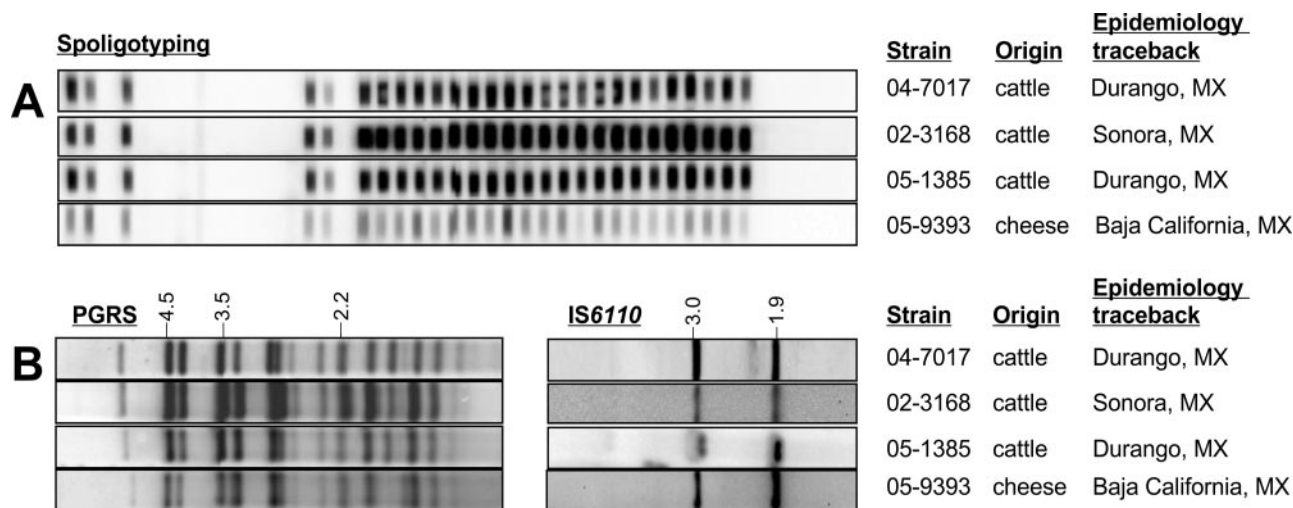


FIG. 1. Genotyping results of *M. bovis* isolates recovered from fresh cheese originating from Mexico and from cattle. The strain designation (Strain), the origin of the *M. bovis* isolate, and the state in Mexico that the diagnostic specimen or animal originated from (Epidemiology traceback) are listed for each isolate. (A) Spoligotype patterns of cheese and cattle *M. bovis* isolates. (B) PGRS and IS6110 RFLP patterns of cheese and cattle *M. bovis* isolates. Approximate molecular mass sizes (kbp) are given above each panel.

cused on discrete regions of Mexico and thus may not represent a comprehensive survey of *M. bovis* strains present in this country. An analysis of the IS6110 RFLP patterns indicates that all of these isolates contain a single copy of this transposable element, as evidenced by two fragments of approximately 3.6 and 1.9 kb in size. This is similar to approximately 85% of all *M. bovis* isolates genotyped at NVSL over the last 6 years (N. B. Harris, unpublished data). These data are also consistent with previous studies evaluating *M. bovis* from cattle in Texas and Mexico, in that the majority of animal strains in these studies also carried a single copy of IS6110 and demonstrated a hybridization band of 1.9 kb in size (19, 24). Because spoligotyping and IS6110 RFLP typing is less discriminatory for *M. tuberculosis* complex isolates with few copies of IS6110, PGRS typing was used to further discriminate among isolates. The PGRS-RFLP profile of the *M. bovis* cheese isolate was also highly similar to the three bovine isolates. However, no direct epidemiological link among any of these isolates is available to support the possibility of any of these strains having a common origin.

In summary, the recovery of *M. bovis* from fresh cheese suggests that human infection through the consumption of unpasteurized dairy products is possible. It also supports the epidemiological conclusions from recent outbreaks that milk products may serve as a reservoir for *M. bovis* transmission to at-risk human populations residing in the United States. However, it should be noted that this survey was not intended to be a systematic study of the recovery of *M. bovis* or other food-borne pathogens in cheese originating from Mexico and thus it is difficult to accurately assess the true impact on public health from this data. Therefore, it is recommended that a more structured study be undertaken, in which the prevalence of *M. bovis* in the animal population within a specific geographic location is examined in conjunction with the recovery of this pathogen from dairy products manufactured within the same region. Nonetheless, *M. bovis* transmission appears to be an important emerging public health concern and will be best

addressed by a collaborative effort between federal and state agencies in both the United States and Mexico.

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